

Transgenic Animal Technology: Technique and Its Application to Improve Animal Productivity

Fahimi Mohammed¹ Tilaye Shibbiru² Ashenafi Mengistu¹ Fanos Tadesse¹

1. College of Veterinary Medicine and Agriculture, Addis Ababa University

2. School of Veterinary Medicine, Wolaita Sodo University

Abstract

Animals with manipulated genetic material (carrying recombinant DNA) are known as transgenic animals. Transgenic technology provides a method to rapidly introduce new genes into animals without cross breeding. It is a powerful technique for studying fundamental problems of mammalian development. Transgenic technology has been developed and found perfect in the laboratory on mice. The three most common gene transfer techniques namely: DNA microinjection, ES-cell mediated and *Retrovirus* mediated gene transfer are the most important to have enabled to produce transgenic cattle, sheep, goat, pig and other animals. Transgenic animals have the potential of agricultural applications like improved growth rate and carcass composition, improved resistance to disease, increased milk yield, improved wool production and so on. The scientific outlook of right and wrong opinions about transgenic animals is called ethics of transgenic animals. These ethical and animal welfare issues surround transgenic animal technology and be only minimized or avoided through awareness creation about the merit of this technology.

Keywords: Animal productivity, Gene transfer, Recombinant DNA, Technique, Transgenic

INTRODUCTION

For a long, animal breeders have strived to improve productive performance traits such as: growth, milk yield and feeding efficiency. Though phenotypic selective breeding was the best practice to enhance superior genetic traits in farm animals, genetic improvements have greatly increased productivity of many domestic animal (Kumar, 2001). Prior to the development of molecular genetics, the regulation and function of the mammalian genes was through the observation of inherited characteristics or spontaneous mutations. Long before Mendel and any molecular genetics knowledge, selective breeding was a common practice among farmers for the enhancement of chosen traits (Vithani, 2008).

A transgenic animal is defined as an animal which is altered by the introduction of recombinant DNA through human interventions. Transgenesis refers to insertion of cDNA (Complimentary Deoxyribonucleic Acid) made from specific mRNA (Messenger Ribonucleic Acid) into cells. The techniques are generally related to the direct manipulation of DNA oriented to the expression of particular genes (Hugon, 2006; Rossana and Cristina, 2010). The contributions of Developmental Biology and Genetic Engineering permitted rapid development of the techniques for the creation of transgenic animals (Rastogi, 2006).

The first gene transfer into mouse using isolated DNA revealed that the generation of animals stably harboring foreign DNA and having modified phenotypic properties was possible (Gurdon and Ruddle, 1981). Some methods of DNA transfer allow random gene addition and targeted gene integration via homologous recombination or gene replacement thus, causing mutation. Targeted mutation refers to a process whereby a specific gene (removal of a gene or part of a gene) is made nonfunctional (knocked-out) or less frequently made functional (knocked-in). These methods do not create new species, but only offer tools for producing new strains of animals that carry novel genetic information (Bagle *et al.*, 2013).

The only current routine use of transgenic animals, primarily mice, is in the area of human disease research. However, potential agricultural applications of such genetic engineering include: improved feed use and faster growth, more resistance to disease, producing more meat that is leaner or that has more of some other desirable quality and possibly even animal waste that is more environmentally benign (Cowan, 2006).

Biotechnology has advanced to a level where it is generally feasible to make particular changes to the genome and, therefore, to the expressed characteristics of living organisms. A transgenic animal is the one that carries a foreign gene (recombinant DNA) that has been deliberately inserted into its genome. In addition to a structured gene, the DNA usually includes other sequences to enable it to be incorporated into DNA of the host and to be expressed correctly by the host (Rastogi, 2006; Purohit, 2010). Transgenic animals are modified in the laboratory to amplify desired characteristics which are beneficial to mankind (Chrenek and Makarevich, 2008).

The world's first transgenic organism was an *E. coli* bacterium transformed with plasmid DNA (Cohen *et al.*, 1973). This technology was extended into animals shortly after 1974, with the first transgenic animal, a mouse containing SV-40 viral DNA fragments (Jaenisch and Mintz, 1974). However, the SV-40 transgene in this case were not actually expressed in the animal. The first transgenic animal expressing its transgene was a mouse containing a cloned growth hormone gene under the control of a metallothionein promoter (Palmiter *et al.*, 1982).

Production of transgenic livestock provides a method to rapidly introduce new genes into cattle without cross breeding. There are different methods used in production of transgenic animals. The major ones are micro-injection, embryonic stem-cell mediated, *Retrovirus* mediated, sperm mediated, transposon mediated and nuclear transfer (Wheeler, 2007). The challenge in creating transgenic animals is to ensure that the transgene is on at the right time and in the right tissue. To be functional, the integrated gene must be expressed and regulated appropriately, thus the gene to be transferred must be accompanied by the appropriate promoter and regulatory sequences. Some genes require an enhancer that may be located far from the promoter. The engineering of organisms requires fusion of the correct promoter or enhancer and gene-coding sequence. The construct must be incorporated into the chromosome where gene expression is regulated. There have been two basic strategies employed in these efforts. The first involves the production of transgenic animals which enhances the animal's value as production trait and enhances the animal value as a producer of conventional animal product. The second strategy in producing transgenic animals is to generate animals that biosynthesize a commercial product other than the conventional animal product (Sasidhara, 2006). Generally, this technology covers the identification and manipulation of genes and their products and its applications in domesticated animals (Taylor and Francis, 2011). Therefore, the objective of this paper is to review on major applications of transgenic animal technology to improve animal productivity.

BASIC TECHNIQUES OF TRANSGENIC ANIMAL TECHNOLOGY

Construction of transgene

Cloning transgene DNA that will be inserted in the recipient is necessary to amplify specific short segments of DNA using either polymerase chain reaction (PCR) or vectors (plasmid DNA or viruses). DNA isolation, cutting and transfer of specific DNA pieces, corresponding to specific important candidate genes can be done (Klug and Cummings, 2002), then DNA ligase ligates DNA fragments into cloning vectors (Anil and Neha, 2005). The construction of a transgene requires the assembly of a few main parts. The transgene must contain a promoter, which is a regulatory sequence that will determine where and when the transgene is active, an exon, a protein coding sequence (usually derived from the cDNA for the protein of interest), stop sequence, intron, poly (A) and enhancer sequences (Clark *et al.*, 2011). A cloning strategy should be clearly planned with a good understanding of each functional element so as to obtain the expected expression of a transgene. Well-defined expression in a cell line is certainly a good indicator that a transgene was properly constructed, although this is not guarantee for transgene expression *in vivo*. A transgene must be excised out of the vector backbone prior to gene transfer. Unnecessary sequences from a plasmid vector (derived from prokaryotic sequences) should be removed as much as possible to increase the probability of transgene expression, although the expression seems unaffected by up to 100 extra bases (Cornel, 2007).

A Promoter sequences are isolated from upstream regions of endogenous mammalian genes and includes a transcriptional start site and transcription regulatory sequences. A protein coding sequence is usually a full-length cDNA derived from the RNA of a gene of interest. This sequence normally contains a translational start codon (ATG), a translational stop codon (UGA, UAG, UAA) and a Kozak sequence upstream of the start codon so that the ribosome can scan and recognize the proper translation start and stop sites on mRNA (Kozak, 1987). Intron in a transgene construct leads to significantly greater transgene expression (Brinster *et al.*, 1988). The intron seems to have important effects on mRNA stabilization and the efficient translocation from nucleus to cytoplasm (Huang and Gorman, 1990). An examples of introns known to have such characteristic are the rabbit beta-globin and the *simian virus 40* (SV40) introns. The construct contain transcriptional stop signals that include the poly (A)-addition sequence (AAUAAA), termination sequences and enhancer sequences which act in position and orientation independently to control the level and pattern of gene expression (Sheets *et al.*, 1987).

Transgene transfer techniques

The establishment of stable transgenic animals implies that the foreign DNA is present in gametes or one-cell embryos to allow its transmission to progeny. To reach this goal, the foreign gene can be transferred using different methods according to animal species (Vithani, 2008). The major ones are micro-injection, embryonic stem-cell mediated, *Retrovirus* mediated, sperm mediated, transposon mediated and nuclear transfer (Wheeler, 2007).

DNA microinjection

Gene transfer by microinjection is the predominant method used to produce transgenic farm animals. This method implies a superovulation of the female egg donors by injection of pregnant mare serum gonadotropin that causes her to release three to four times as many eggs. The eggs are either fertilized *in vitro* or *in vivo* (Pinkert *et al.*, 2001). When fertilization occurs *in vivo*, the fertilized eggs can be taken by euthanizing the female and removing her oviducts. Eggs taken early in development will contain the male and female pronuclei that have not yet fused. Then cloned transgene solution is microinjected into pronuclei using an extremely fine

glass pipette (Pinkert *et al.*, 2001). The male pronucleus is usually chosen for microinjection due to its slightly larger size and its close proximity to the periphery of the egg. In non-mammalian species, the pronuclei cannot be visualized and DNA must be injected into the cytoplasm (Chourrout *et al.*, 1986).

After the pronuclei fuse into one nucleus, producing the zygote, the injected embryos are implanted into a pseudopregnant foster mother. The pseudopregnant foster mother (recipient female or surrogate mother) is made pseudopregnant by either injecting her with hormones or by mating her with vasectomized males which causes a false pregnancy and allows the uterus to receive the egg. The process stimulates the reproductive system, preparing the female's body for transplantation (Krimpenfort *et al.*, 1991).

However, the success rate of producing transgenic animals individually by this method is very low since the insertion of DNA results in a random process and it may be more efficient to use cloning techniques to increase their numbers. The mouse was the first animal to undergo successful gene transfer using this method (Vithani, 2008).

The direct DNA microinjection into the pronuclei of embryos was the first technique which led to regular and relatively easy success in mammals. But it remains inefficient in the laboratory fish *medaka* as well as in *xenopus* and chicken. In these species, foreign DNA usually does not integrate into the genome of the animals. In insects (*Drosophila*) and worms foreign DNA is injected into gonad syncytium (Louis, 2002). A major advantage of this method is its applicability to a wide variety of species (Rastogi, 2006).

Embryonic stem cell mediated gene transfer

This method involves isolation of totipotent stem cells (stem cell that can develop into any type of specialized cell) derived from early pre-implantation embryos. ES-cells are isolated from the inner cell mass of donor blastocysts of early embryos and can be cultured *in vitro* prior to transfection with a specific gene. The cultured pluripotent ES-cells are transfected with the appropriate transgene construct by a suitable transfection technique (preferably by homologous recombination). Transfected ES-cells are identified and selected, generally by employing a selectable marker gene and are cloned. Transformed ES-cells are microinjected into animal blastocysts so that they can become established in the somatic and germ-line tissues. They are then passed onto successive generations by breeding founder animals. Stable transgenic lines are obtained by crossing founder animals that have the gene in their germ cells. The embryos co-cultured and microinjected with transfected ES-cells are transferred into surrogate mother where they complete their development. At present, the ES-cells method is most successful with mice, because mouse ES-cells are pluripotent and when integrated into blastocysts, can divide and differentiate in the mouse embryo (Sasidhara, 2006).

Retrovirus mediated gene transfer

Retrovirus is a virus that carries genetic material in the form of RNA rather than DNA. In this method, *retroviruses* are used as vector to transfer genetic material into the host cell. They do not have the capacity to auto replicate and they have to be integrated stably in the genome of the cells they infected to replicate. Besides, they also have an enzyme called *reverse-transcriptase* which can make DNA from RNA and can easily be altered so that they will not destroy the host cells that they are to invade. This property of *retroviruses* is being implemented to integrate foreign genes. For this purpose, the experimenters remove the genes from the genome of *lentiviruses* (a category of *retroviruses*) and replaced by the genes of interest. Next they microinject the virus into the embryo and let it infect the embryo with the transgene. After that the embryo is allowed to grow to the blastocyst stage and is transplanted into the surrogate mother (Sasidhara, 2006). This method has proved highly efficient in several species including mammals (Pfeifer, 2006) and birds (Lillico *et al.*, 2007).

Use of transposons

Transposons are natural DNA sequences present in the genome of most species and which have the capacity to auto replicate and integrate in multiple sites. In several species, especially non mammals, foreign DNA injected in embryo cytoplasm becomes very rarely integrated in the genome. This is the case for *medaka*, *Drosophila*, chicken and silk worms. In order to enhance the frequency of integration, scientist use Transposons which is a DNA sequences which contain at least one gene coding for a *transposase* which can trigger integration (Houdebine, 2002). Foreign genes can be introduced into transposons *in vitro*. The recombinant transposons may then be microinjected into one day old embryos. The foreign gene becomes integrated into the embryos. All the transgenic insects are being generated by using transposons as vectors. Transposons also proved to be efficient to generate transgenic fish, chicken and mammals (Ding *et al.*, 2005).

Sperm mediated gene transfer

The finding that mature spermatozoa act as vectors of genetic materials, not only for their own genome, but also for exogenous DNA molecules, has suggested a strategy for animal transgenesis. This method appeared difficult to use due to a frequent degradation of DNA (Smith and Spadafora, 2005). Transgenic mice and rabbits were

obtained by incubating sperm with DNA in the presence of DMSO (*Dimethyl Sulfoxide*) and by using conventional *in vitro* fertilization (Shen *et al.*, 2006). The method has been greatly improved, mainly by using ICSI (Intracytoplasmic Sperm Injection). This technique, which consists of injecting sperm into the cytoplasm of oocytes, is currently used for *in vitro* fertilization in humans. To transfer genes, sperms from which plasma membrane have been damaged by freezing and thawing were incubated in the presence of the gene of interest and further used for fertilization by ICSI. This method has proved efficient in mice (Moreira *et al.*, 2007) and pigs (Yong *et al.*, 2006).

Sperm precursors may also be used for gene transfer. In the mouse, *lentiviral* vectors can be injected into seminal tubules to infect spermatogonia which become mature to give sperm harboring the foreign gene and generate transgenic animals (Readhead *et al.*, 2003).

The sperm cells have the capacity to bind naked DNA or bound to vesicles like liposomes (Lavitrano *et al.*, 1989; Chang *et al.*, 2002). These sperm cells are in turn used for introducing exogenous DNA into oocytes either through *in vitro* fertilization or artificial insemination. The sperm mediated gene transfer successfully has carried out in cattle (Sperandio *et al.*, 1996).

Gene transfer by nuclear transfusion

Somatic cell nuclear transfer (SCNT) is a technique for cloning. The nucleus is removed from a healthy egg. The enucleated egg becomes the host for a nucleus that is transplanted from another cell, such as a skin cell. The resulting embryo can be used to generate embryonic stem cells with a genetic match to the nucleus donor or can be implanted into a surrogate mother to create a cloned individual, such as Dolly the sheep. Cloning by nuclear transfer from adult somatic cells is a remarkable demonstration of developmental plasticity. When a nucleus is placed in oocyte cytoplasm, the changes in chromatin structure that govern differentiation can be reversed, and the changed nucleus can control the development of oocyte to term. Dolly was cloned by SCNT with a nucleus from a cultured mammary gland cell (Wilmut *et al.*, 1997).

APPLICATIONS OF TRANSGENIC ANIMALS TO IMPROVE ANIMAL PRODUCTIVITY

There are numerous potential applications of transgenic technology to develop new or altered strains of agriculturally important livestock. Practical applications of transgenic in livestock production include improved milk production and composition, increased growth rate, improved feed utilization, improved carcass composition, increased disease resistance, enhanced reproductive performance and prolificacy, and altered cell and tissue characteristics for biomedical research and manufacturing (Farrand *et al.*, 2004).

Another promising application of transgenic animals is the potential for the development of animal-based food products that contain functional components that enhance food safety or nutritional value and thereby improve human health. For example, the potential to decrease the amount of deleterious fats and cholesterol in milk or increase the prevalence of beneficial fatty acids in pork tissue has been demonstrated and is consistent with dietary recommendations for cardiovascular health (James *et al.*, 2010). Transgenic technology could provide a method to transfer the nutritionally beneficial traits to other food stuffs (Wheeler, 2007).

Improving milk production and composition

Advances in recombinant DNA technology have provided the opportunity either to change the composition of milk or to produce entirely novel proteins in milk. These changes may add value to increase the potential uses of milk. The improvement of livestock growth or survivability through the modification of milk composition requires production of transgenic animals that produce a greater quantity of milk, produce milk of higher nutrient content or produce milk that contains beneficial protein (Noble *et al.*, 2002).

The major nutrients in milk are protein, fat and lactose. By elevating any of these components, we can impact growth and health of the developing offspring. In many production species such as cattle, sheep and goat, the nutrients available to the young may not be limiting. However, milk production in the sow limits piglet growth and therefore, pig production (Hartmann *et al.*, 1984). Methods that increase the growth of piglets during suckling result in an increase in weaning weights, a decrease in the amount of feed needed for the animals to reach market weight. Transgenic alteration of milk composition has the potential to enhance the production of certain proteins and/or growth factors that are deficient in milk (Wall *et al.*, 1991).

The increased expression of a number of these proteins in milk may improve growth, development, health and survivability of the developing offspring. Some of these factors are insulin-like growth factor 1 (IGF-I), epidermal growth factor (EGF), transforming growth factor beta (TGF- β) and lactoferrin (Zhang *et al.*, 2008). Other properties of milk that bear consideration for modifications are those that affect human and animal health. It has been shown that specific antibodies can be produced in genetically engineered animals (Storb *et al.*, 1987).

It should be possible to produce antibodies in the mammary gland that are capable of preventing mastitis in cattle, sheep and goat and MMA (Mastitis-Metritis-Agalactia) in pig and/or antibodies that aid in the prevention of domestic animal or human diseases. Another example is to increase proteins that have

physiological roles within the mammary gland itself such as-lactalbumin (Bleck *et al.*, 1998), lysozyme(Maga *et al.*, 1995, Brundige *et al.*, 2008), lysostaphin or other antimicrobial peptides (Donovan *et al.*, 2005).

The overall result of genetic engineering to modify milk will be the creation of more uses of milk and milk products in both agriculture and medicine. This is truly a “value-added” opportunity for animal production by increasing the concentrations of existing proteins or producing entirely new proteins in milk (Vanberkel *et al.*, 2002).

Enhancing growth rate and carcass composition

The production of transgenic animal has been instrumental in providing new insights into the mechanisms of gene action governing growth. Using transgenic technology, it is possible to manipulate known growth factors, growth factor receptors and growth modulators. Transgenic mice, sheep and pigs have been used to examine postnatal growth of mammals. Based on a recent report in the mouse the myostatin gene is an exceptionally intriguing potential locus for “Knock out” using ES-cells in meat producing species. The loss of the myostatin protein results in an increase in lean muscle mass. Mice lacking this gene have enlarged shoulders and hips. The increased skeletal muscle mass is wide spread throughout the carcass and appears grossly normal. Individual muscle groups from homozygous Knockouts have 2-3 times the weight of control animals. Fat content was comparable in both the wild type and mutant genotypes. Researchers concluded that a large part of the observed increase in skeletal muscle mass was due to muscle cell hyperplasia (Ebert *et al.*, 1988).

Transgenic pigs bearing a human metallothionein promoter/porcine growth-hormone gene construct showed significant improvements in economically important traits such as growth rate, feed conversion and body fat/muscle ratio without the pathological phenotype known from previous growth hormone constructs. Similarly, pigs transgenic for the human insulin-like growth factor-I had 30% larger loin mass, 10% more carcass lean tissue and 20% less total carcass fat. Recently, an important step towards the production of more healthful pork has been made by the creation of the first pigs transgenic for a spinach desaturase gene that produces increased amounts of non-saturated fatty acids. These pigs have a higher ratio of unsaturated to saturated fatty acids in striated muscle, which means more healthful meat since a diet rich in non-saturated fatty acids is known to be correlated with a reduced risk of stroke and coronary diseases (Niemann *et al.*, 2005).

Altering the fat or cholesterol composition of carcass is another benefit that can be delivered via genetic engineering. By changing the metabolism or uptake of cholesterol and/or fatty acids, the content of fat and cholesterol of meats, eggs and cheeses could be lowered. There is a possibility of introducing beneficial fats such as the *omega-3* fatty acids from fish or other animals in livestock (Lai *et al.*, 2006). Receptors such as the low-density lipoprotein (LDL) receptor gene and hormones like leptin are also potential targets that would decrease fat and cholesterol in animal products (Scott and Mattew, 2011).

Generating farm animal resistant to diseases

In most cases, susceptibility to pathogens originates from the interplay of numerous genes; in other words, susceptibility to pathogens is polygenic in nature. Transgenic strategies to enhance disease resistance include the transfer of major histocompatibility-complex genes, T-cell-receptor genes immunoglobulin genes and genes that affect lymphokines or specific disease-resistance genes. Transgenic constructs bearing the immunoglobulin-A (IgA) gene have been successfully introduced into pigs, sheep and mice in an attempt to increase resistance against infections. Attempts to increase *ovine* resistance to *Visna virus* infection via transgenic production of *Visna virus* envelope protein have been reported. An interesting achievement was the production of cattle lacking the prionprotein and this prevent infection and transmission of spongiform encephalopathies like scrapie or bovine spongiform encephalopathy (Richt *et al.*, 2007).

Transgenic dairy cows that secrete lysostaphin in their milk have been produced to address the mastitis issue. Lysostaphin is an antimicrobial peptide that protects mammary gland against *Staphylococcus aureus* infection by killing the bacteria in a dose dependent manner (Kerr *et al.*, 2011). Influenza resistant pigs are also produced after the introduction of mice fibroblast cell lines that contain the protein which is resistant to infection with *influenza virus*. The application of chimeras or NT technology will enable the amplification of beneficial alleles and/or the removal (via gene “knock out”) of undesirable alleles associated with disease resistance or susceptibility. An example is “knocking –out” the intestinal receptor for the k88 antigen. The absence of the antigen has been shown to confer resistance to both experimentally and naturally induced infection of k88-positive *E.coli*. Though scientists are attempting to produce disease resistant animals such as influenza resistant pigs, a very limited number of genes are currently known to be responsible for resistance to disease in farm animals (Vithani, 2008).

The levels of the anti-microbial peptides; lysozyme and lactoferrin in human milk are many times higher than in bovine milk. Transgenic expression of the human lysozyme gene in mice was associated with a significant reduction of bacteria and reduced the frequency of mammary gland infections. Lactoferrin has

bactericidal and bacteriostatic effects, in addition to being the main iron source in milk. These properties make an increase in lactoferrin levels in bovine transgenics a practical way to improve milk quality. Human lactoferrin has been expressed in the milk of transgenic mice and cattle at high levels and is associated with an increased resistance against mammary gland diseases (Niemann *et al.*, 2005).

Disease resistance benefits not only livestock producers and their animals, but consumers also benefit as a result of safer animal products in the market and a reduction in the incidence of humantransmissible diseases such as *avian influenza* (Alison and Davis, 2009).

Improving reproductive performance and fecundity

Several candidate genes have been identified that increase the reproductive performance of farm animals. These included the estrogen receptor (ESR) and the Booroola fecundity (FECB) genes. Introduction of a mutated or polymorphic ESR gene could increase litter size in case of pigs (Rothschild *et al.*, 1994).

The ovulation rate of different breeds of sheep, which are superior in carcass traits and wool production, could be increased by incorporating a single major autosomal gene called booroola fecundity gene (FECB) that increases the prolificacy in sheep (Piper *et al.*, 1985). Each copy of this gene increases ovulation rate by approximately 1.5 ova per cycle (Scott and Mattew, 2011). The estrus symptoms in case of pigs could be enhanced by incorporating a gene from baboons, which make their posterior red (Seidel, 1999).

Improving hair and fiber production

The control of the quality, color, yield and even ease of harvest of hair, wool and fiber for fabric and yarn production has been an area of focus for transgenic manipulation in livestock. The manipulation of the quality, length fineness and crimp of the wool and hair fiber from sheep and goats has been examined using transgenic methods (Scott and Mattew, 2011).

Transgenic methods will also allow improvements to fiber elasticity and strength. In the future transgenic manipulation of wool will focus on the surface of the fibers. Decreasing the surface interactions between fibers could decrease shrinkage of garments made from such fibers (Bawden *et al.*, 1999). Another application of this technology is the efforts to induce sheep to shed their wool at specific times, to alleviate the need for hand shearing of fiber producing animals. Genes such as EGF with inducible promoters have been introduced into sheep. The idea is that when EGF expression is induced, a weak spot is produced in the wool fiber that allows the fleece to be removed easily (Wheeler, 2007).

Generally the aim is to improve sheep for wool production and to modify the properties of the fiber. Because *cysteine* seems to be the limiting amino acid for wool synthesis, the approach is to increase its production through transfer of *cysteine* biosynthesis from bacterial genes to sheep genome (Murray *et al.*, 1999).

Improvements of feed utilization and the modification of animal metabolism

Another great advantage of transgenic animal is also to improve feed efficiency and/or appetite that could profoundly impact livestock production and deliver significant benefits to producers, processors, and consumers. Increased uptake of nutrients in the digestive tract, by alteration of the enzyme profiles in the gut, could increase feed efficiency. The ability to introduce enzymes such as *phytase* or *xylanase* into the gut of species where they are not normally present, such as swine or poultry, is particularly attractive. The introduction of *phytase* would increase the bioavailability of phosphorus from phytic acid in corn and soy products. There is a production of the transgenic pigs expressing salivary *phytase* as early as seven days of age. The salivary *phytase* provided essentially complete digestion of the dietary phytate phosphorus in addition to reducing phosphorus output in waste by up to 75 percent. The use of *phytase* transgenic pigs in commercial pork production could result in significantly decreased environmental phosphorus pollution from livestock (Golovan *et al.*, 2001).

Improving production in aquaculture

Several relevant desired phenotypes have been genetically engineered into fish, including, enhanced growth rate (Rahman *et al.*, 1998; Zhu and Sun, 2000), resistance to bacterial diseases (Yazawa *et al.*, 2006), tolerance to cold temperatures (Wang *et al.*, 1995), improved nutrient use and biocontrol of invasive species (Angulo and Gilna, 2008).

Transgenic fish which contain an exogenous GH gene has been produced. This type of work enabled the study of chronic expression of these hormones on growth in fish. For example, dramatic increases have been shown in growth rate of transgenic *Atlantic salmon* using the gene promoter and growth hormone gene derived from fish species (Hew *et al.*, 1995).

These researchers also indicate that fish used in Aquaculture would be made sterile, thus minimizing the ecological impact due to accidental escape of fish that might be raised in ocean pens. Introduction of *salmonid* GH constructs has resulted in a 5-11 fold increase in weight after one year of growth. This demonstrates that increased growth rate and ultimately increased rate of protein production can be achieved via

transgenic technology (Devlin *et al.*, 2002).

CONCLUSION AND RECOMMENDATIONS

Genetic modification of animals by recombinant DNA technology (transgenesis) entails the introduction of a cloned gene(s) into the genome of a cell that might, after proliferation, be present in the germ lines of developed organisms, so that subsequently it may be possible to establish true breeding lineages. It is a more extreme methodology, but in essence, not really different from cross breeding or genetic selection in its result. One of the most common methods used to produce transgenic animals is the injection of foreign DNA sequence into the pronucleus of recently fertilized zygotes. This allows the DNA to integrate randomly into a chromosome and subsequently be expressed in somatic and germ tissues of the resultant individual. Other methods of DNA transfer are by ES-cell mediated, *Retrovirus* mediated, sperm mediated, transposon mediated and nuclear transfer. There are numerous potential applications of transgenic technology to develop new or altered strains of agriculturally important livestock. Practical applications of transgenic in livestock production include improved milk production and composition, increased growth rate, improved feed utilization, improved carcass composition, increased disease resistance, enhanced reproductive performance and prolificacy and altered cell and tissue characteristics for biomedical research and manufacturing purpose. Even though there are a number of transgenic technologies, the rate at which these technologies are incorporated into production schemes will determine the speed at which we will be able to achieve our goal of more efficiently producing livestock, which meets consumer and market demand.

In light of the above conclusive remarks, the following recommendations are forwarded:

- ☞ A lot of current research should focus on identifying important genes, developing useful promoter sequences to control transgenes and establishing more precise ways to insert the transgene into the recipient.
- ☞ Awareness creation on ethical and animal welfare issues surrounding transgenesis and transgenic animal's product.
- ☞ The manipulation of reproductive processes using transgenic methodologies should be considered as a huge area for investigation in the future.

REFERENCES

- Alison, V. and Davis, U. (2009): Potential effects of biotechnology on animal health and well-being. Applications of Animal Biotechnology in Animal Health, Department of Animal Science, University of California, California, Pp1-5.
- Angulo, E. and Gilna, B. (2008): When biotech crosses borders. *Nature Biotechnol*, 26: 277-282.
- Anil, K. and Neha G. (2005): Genetic Engineering. 1st Edition, Nova Science Publisher Inc, New York, Pp 15-16.
- Bagle, S., Kunkulol, R., Baig, M. and More, S. (2013): Transgenic animals and their application in medicine. *Int. J. Medical Res. And Health Sci.*, 2:107-116.
- Bawden, C., Dunn, S., McLaughlan, C., Nesci, A., Powell, B., Walker, S., Rogers G. (1999): Transgenesis with *ovine* keratin genes: expression in the sheep wool follicle for fibres with new properties. *Trans Res.*, 8:474-476.
- Bleck, G., White, B., Miller, D. and Wheeler M. (1998): Production of *bovine alpha-lactalbumin* in the milk of transgenic pigs. *J Anim Sci.*, 76: 3072-3078.
- Brinster, R., Allen, J., Behringer, R., Gelinas, R. and Palmiter, R. (1988): Introns increase transcriptional efficiency in transgenic mice. *Proc Natl Acad Sci U S A*, 85:836-840.
- Brundige, D., Maga E., Klasing K. and Murray J. (2008): *Lysozyme* transgenic goat's milk influences gastrointestinal morphology in young pigs. *J Nutr*, 138: 921-926.
- Chang, K., Qian, J., Jiang M., Lui, Y., Wu, C., Chen, C., Lai, C., Hsiao, C., Brown, L., Bolen, J., Huang, H., Ho, P., Shih, P., Yao, C., Lin, W., Chen, C., Wu, F., Lin, Y., Xu, J. and wang, K. (2002): Effective generation of transgenic pigs and mice by linker based sperm-mediated gene transfer. *BMC Biotechnol*, 2: 1-13.
- Chourrout, D., Guyomard, R. and Houdebine, L. (1986): High efficiency gene transfer in rainbow trout (*salmo gairdneri* rich) by microinjection into egg cytoplasm. *Aquaculture*, 51: 143-150.
- Chrenek, P. and Makarevic, A. (2008): Transgenic rabbits – production and application. *Slovak J. Anim. Sci.*, 41: 113-120.
- Clark, A., Archibald, M., McClenaghan, J., Simons, R. and Wallace C. (2011): "[Transgene Design](#)". Whitelaw Philosophical Transactions: *Bio Sci*, 339: 1288.
- Cohen, S., Chang, A., Boyer, H., and Helling, R. (1973): Construction of biologically functional bacterial plasmids in vitro. *Proc Natl Acad Sci USA*, 70: 3240-3244.
- Cornel, M. (2007): Molecular Biology and Genomics. 1st Edition, Elsevier Inc, San Diego, Pp 116-218.
- Cowan, T. (2006): Biotechnology in Animal Agriculture: Status and Current Issues. CRS Report for

- Congress. Congressional research service, Pp 1-22.
- Devlin, R., Yesaki, T., Biagi, C., Donaldson, E., Swanson P. and Chan W. (2002): Extraordinary salmon growth. *Nature*, 371:209-210.
- Ding, S., Wu, X., Li, G., Han, M., Zhuang, Y. and Xu, T. (2005): Efficient transposition of the piggyBac (PB) transposon in mammalian cells and mice. *Cell*, 122:473-483.
- Donovan, D., Kerr, D., and Wall R. (2005): Engineering disease resistant cattle. *Trans Res.*, 14:563-567.
- Ebert, K., Low, M., Overstrom, E., Buonoma, F., Roberts, T., Lee, A., Mandel, G. and Goodman R. (1988): A Moloney MLVRAT somatotropin fusion gene produces biologically active somatotropin in a transgenic pig. *Mol Endocrinol*, 2: 77-283.
- Farrand, S., Wheeler M. and Widholm J. (2004): Animal and Plant Transformation: The Application of Transgenic Organisms in Agriculture. <http://www.aces.viuc.edu/vista/html-pubs/irpspm91/transfer.html> Accessed on 07 March, 2016.
- Golovan, S., Meidinger, R., Ajakaiye, A., Cottrill, M., Wiederkehr, M., Barney, D., Plante, C., Pollard, J., Phillips, J. and Forsberg C. (2001): Pigs expressing *salivary phytase* produce low-phosphorus manure. *Nature Biotechnol*, 19:741-745.
- Gurdon, J.W. and Ruddle, F.H (1981). Integration and stable germline transmission genes injected into mouse pronuclei. *Science*, 214: 1244-1246.
- Hartmann, P., McCauley, I., Gooneratne, A. and Whitely J. (1984): Inadequacies of sow lactation: survival of the fittest. *Symp Zool Soc Lond*, 51: 301-326.
- Hew, C., Fletcher G. and Davies, P. (1995): Transgenic salmon: tailoring the genome for food production. *J Fish Bio*, 47:1-19.
- Huang, M. and Gorman, C. (1990): Intervening sequences increase efficiency of RNA 3' processing and accumulation of cytoplasmic RNA. *Nucleic Acids Res.*, 18:937-947.
- Houdebine, L.M. (2002). The methods to generate transgenic animals and to control transgene expression. *J. Biotechnol*. 98:145-160
- Hugon, H. (2006): Genetic engineering applications in animal breeding. *Elect J Biotechnol*, 9:158-162.
- Jaenisch, R. and Mintz, B. (1974): *Simian virus 40* DNA sequences in DNA of healthy adult mice derived from pre-implantation blastocysts injected with viral DNA. *Proc. Natl. Acad. Sci. USA*, 71: 1250-1254.
- James, D., Nuradilla, M., Caitlin, A. and Elizabeth A. (2010): Current status of transgenic animal research for human health Applications. *Acta Scientiae Veterinariae*. 38: 627-632.
- Kerr, D., Plaut, K., Bramley, A., Williamson, C., Lax, A., Moore, K. and Wall, R. (2011): *Lysostaphin* expression in mammary glands confers protection against *staphylococcal* infection in transgenic mice. *Nature Biotechnol*, 19: 66-70.
- Klug, W. and Cummings, M. (2002): Concepts of Genetics. 7th Edition, Prentice Hall, New Jersey, Pp 800.
- Kozak, M. (1987): An analysis of 5'-noncoding sequences from 699 vertebrate messenger RNAs. *Nucleic Acids Res.*, 15:8125-8148.
- Krimpenfort, P., Rademakers, A., Eyestone, W., Vander schans, A., Vandebroek, S., Kooiman, P., Kootwijk, E., Platenburg, G., Pieper, F. and Strijker, R. (1991): Generation of transgenic dairy cattle using 'in vitro' embryo production. *Bio/Technol*, 9: 844-847.
- Kumar, H. (2001): Modern Concepts of Biotechnology. Jankpura, VIKAS PVT.LTD. Pp: 252, 256, 273, 281.
- Lai, L., Kang, J., Li R., Wang, J., Witt, W., Yong, H., Hao, Y., Wax, D., Murphy, C., Rieke, A., Samuel, M., Linville, M., Korte, S., Evans, R., Starzl, T., Prather, R. and Dai, Y. (2006): Generation of cloned transgenic pigs rich in *omega-3 fatty acids*. *Nat Biotechnol*, 24:435-436.
- Lavitrano, M., Camaioni, A., Fazio, V.; Dolci, S., Farace, M. and Spadafora, C. (1989): Sperm cells as vectors for introducing foreign DNA into eggs: Genetic transformation of mice. *Cell*, 57: 717-723.
- Lillico, S., Sherman, A., McGrew, M., Robertson, C., Smith, J., Haslam, C., Barnard, P., Radcliffe, P., Mitrophanous, A., Elliot, E. and Sang, H. (2007): Oviduct-specific expression of two therapeutic proteins in transgenic hens. *Proc Natl Acad Sci.*, 104: 1771-1776.
- Louis, H. (2002): The methods to generate transgenic animals and to control transgene. *J Biotechnol*, Pp 145 -160.
- Maga, E., Anderson, G. and Murray J. (1995): The effect of mammary gland expression of human *lysozyme* on the properties of milk from transgenic mice. *J Dairy Sci*, 78: 2645-2652.
- Moreira, P., Pozueta, J., Pérez, M., Valdivieso, F., Gutiérrez-Adán, A. and Montoliu, L. (2007): Improving the generation of genomic-type transgenic mice by ICSI. *Transgenic Res.*, 16:163-168.
- Murray, J. Oberbauer, A. And Mcgloughlin, M. (1999): Transgenic animals in Agriculture. Davis, CABI Publishing, Pp 304.
- Niemann, H., Kues W. and Carnwath J. (2005): Transgenic farm animals: present and future, Department of Biotechnology, Institute for Animal Breeding (FAL), Mariensee, Germany. *Rev. Sci. tech. Off. Int.*, 24: 285-298.
- Noble, M., Rodriguez S., Bleck G., Cook J., Hurley W. and Wheeler M. (2002): Lactational performance of first

- parity transgenic gilts expressing bovine \pm lactalb min in their milk. *J AnimSci*, 80: 1090-1096.
- Palmiter, R., Brinster, R., Hammer, R., Trumbauer, M., Rosenfeld, M., Birnberg, N. and Evans, R. (1982): Dramatic growth of mice that develop from eggs microinjected with *metallothionein-growth hormone fusion genes*. *Nature*, 611–615.
- Pfeifer, A. (2006): *Lentiviral* transgenesis – a versatile tool for basic research and gene therapy. *Curr Gene Ther.*, 6:535–542.
- Pinkert, C., Johnson, L., Irwin, M., Wong, S., Baetge, E., Wolfe, D., Simpkins, S., Owsley, W. and Bartol, F. (2001): Optimization of superovulation and fertilization protocols in the production of transgenic swine. *AdvReprod*, 5: 45-53.
- Piper, L., Bindon, B. and Davis, G. (1985): The single inheritance of the high litter size of the *Boorola Merino*. In: Land, Robinson, D. (ed): *Genetics of Reproduction in Sheep*. Butterworths, London. Pp 115-125.
- Purohit, S. (2010): *Biotechnology: Fundamental and Applications*. 4th Edition. Joldhpur, Student Edition, India. Pp 421, 432,436.
- Rahman, M., Mak, R., Ayad, H., Smith, A. and Maclean, N. (1998): Expression of a novel piscine growth hormone gene results in growth enhancement in transgenic tilapia (*Oreochromis niloticus*). *Transgenic Res.*, 7: 357-369.
- Rastogi, S. (2006): *Introduction to Biotechnology*, New Delhi. Bangalore, CBS, India. Pp: 173-182.
- Readhead, C., Jarvis, S., Morgan, D. and Winston, R. (2003): Male germ cells: manipulating their genome. In *Transgenic Animal Research Conference IV*, Tahoe City, USA, Pp 11.
- Richt, J., Kasinathan, P., Hamir, A., Castilla, J., Sathiyaseelan, T., Vargas, F., Matsushita H., Koster, J., Kato, S., Ishida, I., Soto, C., Robl, J. and Kuroiwa, Y. (2007): Production of cattle lacking prion protein. *Nature Biotechnol*, 25: 132-138.
- Rossana, D. and Cristina, G. (2010): Towards the recombinant drug. *EurMolBiol Lab*, 4:34-35.
- Rothschild, M., Jacobson, C., Vaske, D., Tuggle, C., Sasaki, S., Eckardt, G. and McLaren, D. (1994): A major gene for litter size in pigs. In: *Proceedings of the Fifth World Congress, Genet Applied to Livestock Production*, Pp 225-228.
- Sasidhara, R. (2006): *Animal Biotechnology*, Chennai, MJP, India. Pp 113-166.
- Scott, G. and Mattew, B. (2011): Genetically engineered animals and public health. *Compelling Benefits for Health Care, Nutrition, the Environment and Animal Welfare*. Pp 22-30.
- Seidel, G. (1999): The future of transgenic farm animals. In: *Transgenic Animals in Agriculture*, Murray J. (ed): CAB International. Pp 269-282.
- Sheets, M., Stephenson, P. and Wickens, M. (1987): Products of in vitro cleavage and polyadenylation of *simian virus 40* late pre-mRNAs. *Mol Cell Biol.*, 7:1518–1529.
- Shen, W., Li, L., Pan, Q., Min, L., Dong, H. and Deng, J. (2006): Efficient and simple production of transgenic mice and rabbits using the new DMSO-sperm mediated exogenous DNA transfer method. *MolReprodDev*, 73:589–594.
- Smith, K. and Spadafora, C. (2005): Sperm mediated gene transfer: applications and implications. *BioEssays*, 27:551–562.
- Sperandio, S., Lulli, V., Bucci, M., Forni, M., Maidone, B., Spadafora, C. and Lavitrano, M. (1996): Sperm-mediated DNA transfer in *Bovine* and *Swine* species. *AnimBiotechnolo*, 7: 59-77.
- Storb, U. (1987): Transgenic mice with immunoglobulin genes. *Annu Rev Immunol*, 5: 151-174.
- Taylor, M. and Francis, J. (2011): Recent advances in transgenic animals to overcome impediments of standard antibody technologies to improve the generation of specific antibodies. *MAbs J.*, 5: 431-439.
- Vanberkel, P., Welling, M., Geerts, M., Veen, H., Ravensbergen, B., Salajeddine M., Pauwels, E., Pieper, F., Nuijens, J. and Nibbering P. (2002): Large-scale production of recombinant human lactoferrin in the milk of transgenic cows. *Nature*, 20: 484-487.
- Vithani, N. (2008): Definition and Introduction to the Transgenic System: <http://www.bookrags.com/research/transgenicanimalgene/>. Accessed on 11 March, 2016.
- Wall, R., Pursel, V., Shamay, A., McKnight, C., Pittius, R. and Henninhausen, L. (1991): High-level synthesis of a heterologous milk protein in the mammary glands of transgenic swine. *ProcNatAcadSci USA*, 88: 1696-1700.
- Wang, R., Zhang, P., Gong, Z. and Hew C. (1995): Expression of the antifreeze protein gene in transgenic goldfish (*Carassius auratus*) and its implication in cold adaptation. *Mol Marine Bio and Biotechnol* 4: 20-26.
- Wheeler, M. (2007): Cloning and Transgenic Animals for Agriculture. <http://www.Geraembryo.com.br/t.technicos/p4/matthew-wheeler.pdf> Accessed on 8 March, 2016.
- Wilmut, I., Schnieke A., Mcwhir J., Kind A. And Campbell K. (1997): Viable offspring derived from fetal and adult mammalian cells. *Nature*, 385: 810-813.
- Yazawa, R., Hirono, I. and Aoki, T. (2006): Transgenic zebrafish expressing chicken lysozyme show resistance

- against bacterial diseases. *Trans. Res.*, 15: 385-391.
- Yong, H., Hao, Y., Lai, L., Li, R., Murphy, C., Rieke, A., Wax, D., Samuel, M. and Prather, R. (2006): Production of a transgenic piglet by a sperm injection technique in which no chemical or physical treatments were used for oocytes or sperm. *MolReprodDev*, 73:595–599.
- Zhang, J., Li, L., Cai, Y., Xu, X., Chen, J., Wu, Y., Yu, H., Yu, G., Liu, S., Chen, A. and Cheng, G. (2008): Expression of active recombinant human *lactoferrin* in the milk of transgenic goats. *Protein Expr Purif*, 57: 127-135.
- Zhu, Z. and Sun, Y. (2000): Embryonic and genetic manipulation in fish. *Cell Res.*, 10: 17-27.